

Original paper

First record of *Xiphinema globosum* Sturhan, 1978 (Nematoda: Longidoridae) from Croatia

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Accepted: 7 June 2023 / Published online: 13 July 2023

Summary. The discovery of a population of *Xiphinema globosum* in a soil sample collected in 2012 at Horvatove stube, Medvednica Mt. represents the first recording of this species in Croatia. This population is briefly described, illustrated, and morphometric data for females, males and four juvenile developmental stages are presented. Previous findings for this species are discussed and commented.

Keywords: geographic distribution, Horvatove stube, juvenile developmental stages, Medvednica Mt., morphology, morphometrics, *Taxus baccata*.

INTRODUCTION

Xiphinema globosum was originally described from Germany (Sturhan 1978) and has also been recorded from Slovenia (Barsi 1992), from few localities in Italy, mainly in the north (Roca and Lamberti 1994), and from Spain (Cantalapiedra-Navarrete et al. 2011).

A soil sample collected in the rhizosphere of the common yew, *Taxus baccata* L., on April 13, 2012 at the site of Horvatove stube on the Medvednica Mt. in Croatia, contained a population of *X. globosum*.

The aim of the present study was to extend knowledge of the geographical distribution, morphology, and intraspecific variability of *X. globosum*, as well as to provide illustrations of adults and juvenile developmental stages.

MATERIAL AND METHODS

Nematodes were extracted from soil sample using Cobb's wet sieving technique. Specimens were killed by hot FP 4-1 and transferred to glycerin by a slow evaporation method and mounted on permanent microscope slides. Measurements were made with an ocular micrometer, with the exception of body, pharynx and tail lengths for all developmental stages, or replacement odontostyle length for juvenile stages. These characteristics were drawn using a drawing tube on an Olympus CX31 microscope at the appropriate magnification. Drawings were scanned and measurements were taken from the scanned drawings using Digimizer Version 4.6.1 software for digital measurements. Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with an AxioCam MRc 5 digital camera.

RESULTS

Xiphinema globosum Sturhan, 1978

(Figs 1-5)

Measurements
See Table 1.

Description

Female. Habitus as open C after fixation; body cylindrical, tapering very gradually towards the extremities. Lip region slightly rounded, offset from the rest of the body by a shallow depression. Amphid pouches stirrup shaped with a wide aperture located just anterior to the demarcation line. Odontostyle, odontophore and guide sheath typical of the genus. Pharynx dorylaimoid with the basal enlarged portion occupying about 23.7% of its total length and measuring 106 (93-120) μm long and 27 (26-30) μm wide. Reproductive system amphidelphic, with equally developed branches. Pseudo-Z-organ with 4-5 sclerotized, irregularly shaped bodies of various sizes. Vulva a tranverse slit; vagina perpendicular to long body axis, reaching from 35.1% to 51.5% of the corresponding diameter. Prerectum (n = 4) 391 (343-418) μm or 8 (7.1-8.7) anal body diameter long. Tail short, convex-conoid to hemispherical, with broadly rounded tip; 2-3 (4 in one specimen) caudal pores on each side.

Male. Generally similar to female, except for the reproductive system, with the posterior region of the body more coiled. Spicules well sclerotized, massive. One precloacal pair and 3-6 ventromedian supplements present. Tail similar to that of female; 3-5 caudal pores on each side.

Juveniles. Clearly separated into four developmental stages (Fig. 4). They resemble adults except for smaller size. Tail shape: in J1 regularly conoid with cuticular extension, slightly curved ventrally; in J2 regularly conoid with cuticular extension, slightly curved ventrally in posterior region; in J3 and J4 conoid with broadly rounded tip, without cuticular extension (Fig. 3).

Individual increase in the length of replacement odontostyle in relation to functional odontostyle in individuals in four juvenile developmental stages indicate that this increase was 22.8 (13.1-43.9)% in J1, 25.9 (18.5-32.0)% in J2, 21.8 (9.4-31.3)% in J3, and 19.4 (12.3-28.2)% in J4 (Fig. 5).

DISCUSSION

Since 1978, when Sturhan described *X. globosum* from Germany, three records of this species have been published. Barsi (1992) from Slovenia, Roca and Lamberti (1994) from Italy, and Cantalapedra-Navarrete et al. (2011) from Spain. The findings from Croatia presented in this paper represent the fourth record.

All populations originate from natural habitats. In Germany: Haunstetter Wald nature reserve south of Ausburg. Spruce stands in the alluvial forest area west of the Lech, with sparse undergrowth of *Angelica* sp., *Aegopodium* sp., *Asarum* sp., *Daphne* sp., *Berberis* sp. and Gramineae. In Slovenia: rhizosphere of European spruce, *Picea excelsa* (Lam.) Lk, at Podbrezje, and in the rhizosphere of common bracken, *Pteridium aquilinum* (L.) Kuhn, at Idrija. In Italy: elm forest at Artegna (Udine) and spruce and elm woods at Resiutta (Udine). In Spain: sandy soil around the roots of black alder, *Alnus glutinosa* (L.) Gaertn., and river bank grapevine, *Vitis riparia* Michx, in the canuto of Valdeinfierno in Los Alcornocales Regional Park, Alcalá de los Gazules, Cádiz Province, southern Spain. In Croatia: in the rhizosphere of the common yew, *Taxus baccata* L., at Horvatove stube, Medvednica Mt.

Adults (females and males) were presented in all papers, but juvenile developmental stages have been reported in only four, including the original description (Table 2). Considering the number of individuals whose morphological and morphometric data were presented in these papers, their number is modest, especially with respect to the juvenile

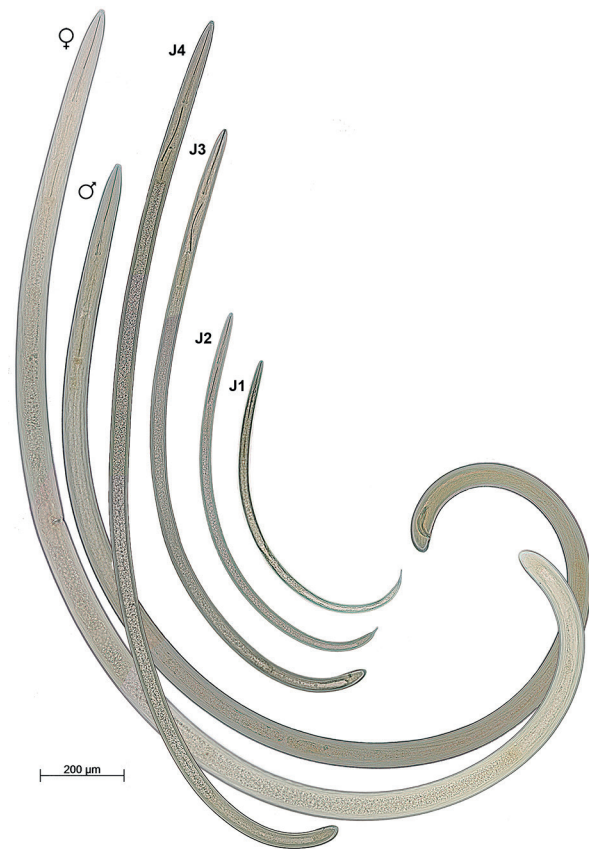


Fig. 1. *Xiphinema globosum* (Horvatove stube, Medvednica Mt., Croatia). Entire body of female (♀), male (♂) and four juvenile developmental stages (J1-J4).

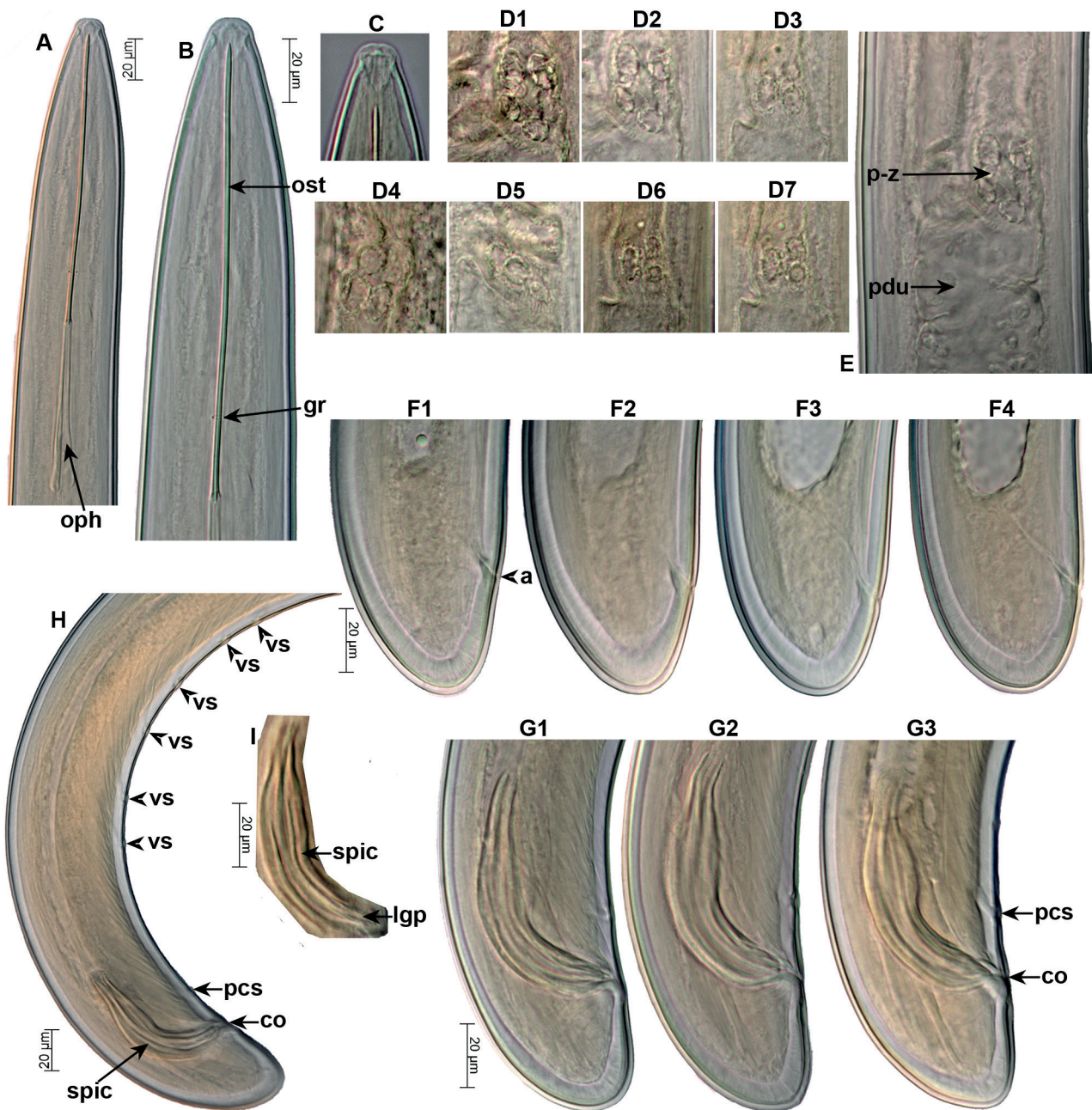


Fig. 2. *Xiphinema globosum* (Horvatove stube, Medvednica Mt., Croatia). **A-B**, Anterior region of female in lateral optical view (gr = guiding ring; ost = odontostyle; oph = odontophore); **C**, Female lip region with amphid; **D1-D7**, Pseudo-Z-organ in detail; **E**, Detail of the female posterior genital branch (p-z = pseudo-Z-organ; pdu = *pars dilatata uteri*); **F1-F4**, Female tail region (a = anus); **G1-G3**, Male tail region (co = cloacal opening; pcs = precloacal supplements); **H**, Male posterior region (co = cloacal opening; pcs = precloacal supplements; spic = spicules; vs = ventromedian supplements); **I**, Spicule (lgp = lateral guiding piece). (Scale bars: A, H = 20 μ m; B-G, I = 20 μ m.)

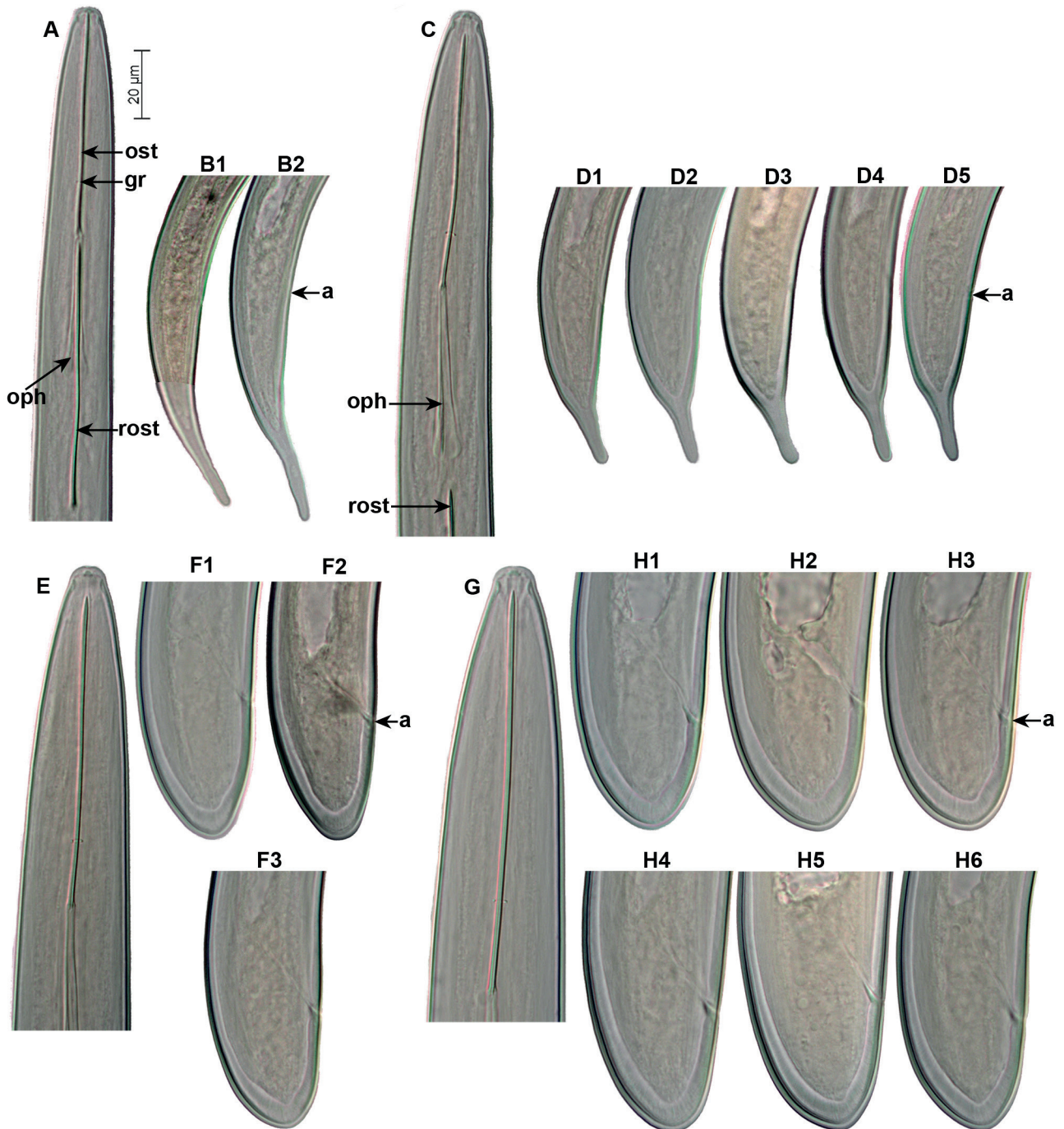


Fig. 3. *Xiphinema globosum* (Horvatove stube, Medvednica Mt., Croatia), juvenile developmental stages J1-J4. **J1:** **A**, Anterior body region (ost = odontostyle; gr = guiding ring; oph = odontophore; rost = replacement odontostyle); **B1-B2**, Tail region (a = anus); **J2:** **C**, Anterior body region (oph = odontophore; rost = replacement odontostyle); **D1-D5**, Tail region (a = anus); **J3:** **E**, Anterior body region; **F1-F3**, Tail region (a = anus); **J4:** **G**, Anterior body region; **H1-H6**, Tail region (a = anus). (Scale bar: 20 µm.)

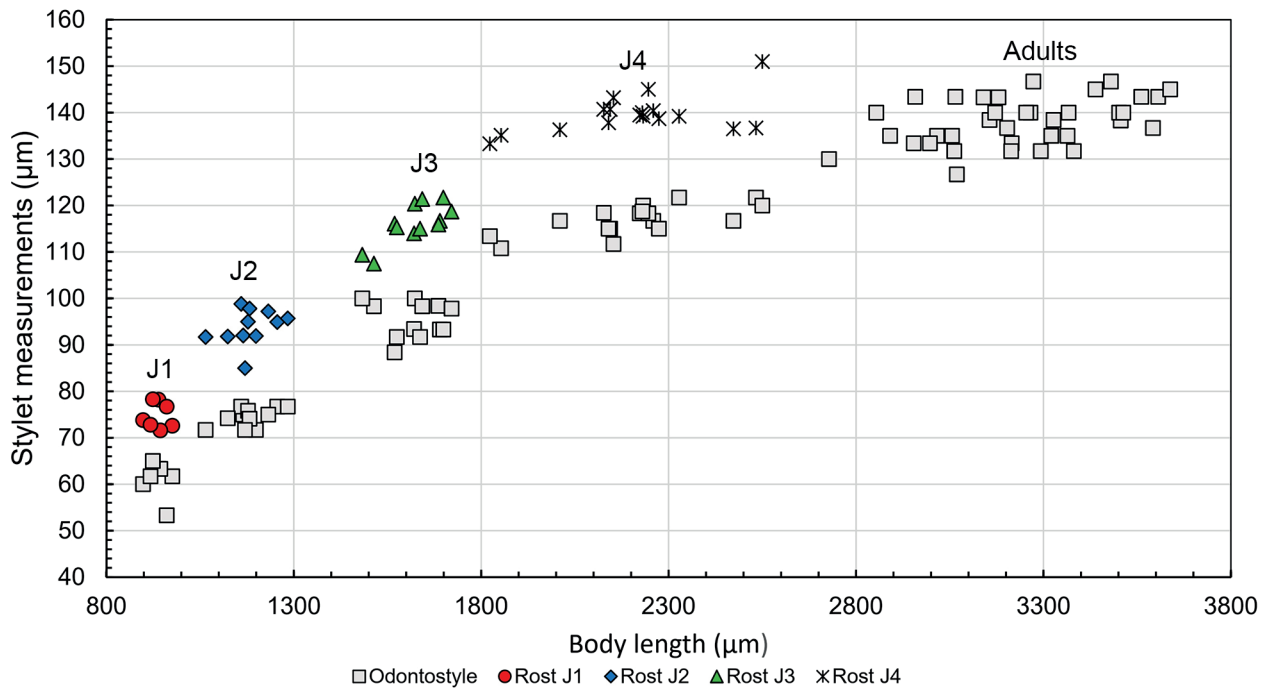


Fig. 4. Scatter diagram plotting the length of stylet measurements vs. body length of individual specimens of the four juvenile stages (J1-J4) and adult females and males of *Xiphinema globosum* (Horvatove stube, Medvednica Mt., Croatia).

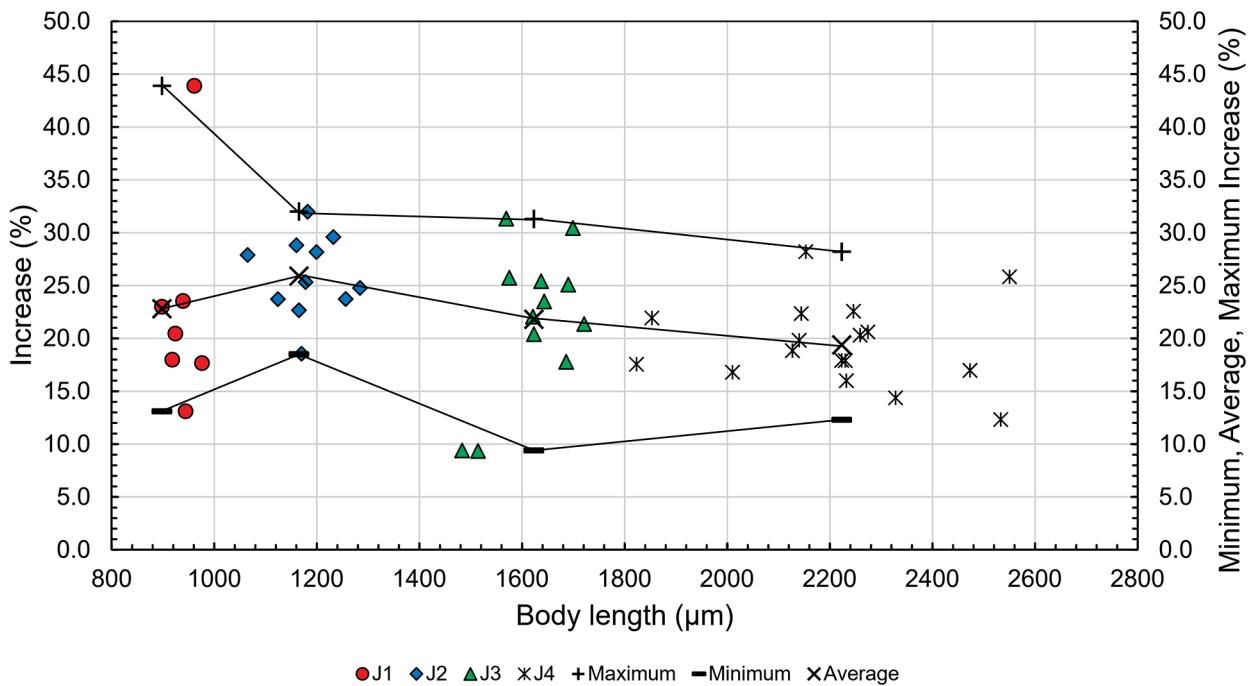


Fig. 5. *Xiphinema globosum* (Horvatove stube, Medvednica Mt., Croatia). Individual increase in length of replacement odontostyle in relation to functional odontostyle in individuals in 4 juvenile developmental stages (J1-J4); minimum, average and maximum individual increase.

stages. It is obvious that in addition to the general similarity that exists between populations, there is also inter-population variability, which is expressed in the variability of some morphometric values, the most significant of which are summarized in Tables 2-5. Looking at the existing variability among populations and bearing in mind their origin, we can consider all of this to be intraspecific variability.

ACKNOWLEDGMENTS

The author would like to thank Dr. Ivo Karaman for the soil sample collected in 2012 at Horvatove stube, Medvednica Mt., Croatia.

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Table 1. Morphometrics of adult and juvenile *Xiphinema globosum* from Croatia. All measurements are presented in μm (except for L) and in the form: mean \pm standard deviation (range)

Locality:	Horvatove stube, Medvednica Mt., Croatia					
Host:	<i>Taxus baccata</i>					
	J1	J2	J3	J4	Female	Male
n	7	11	12	17	18	18
L (mm)	0.94 \pm 0.02 (0.9-0.98)	1.18 \pm 0.06 (1.07-1.28)	1.62 \pm 0.07 (1.48-1.72)	2.21 \pm 0.20 (1.82-2.55)	3.31 \pm 0.24 (2.85-3.64)	3.17 \pm 0.20 (2.73-3.51)
a	38.2 \pm 1.4 (36-39.7)	38.0 \pm 2.0 (35.1-41.7)	42.1 \pm 2.6 (37.1-48.3)	47.1 \pm 2.2 (42.1-51.1)	48.5 \pm 2.4 (43.9-54.1)	47.2 \pm 3.7 (37.8-51.1)
b	3.7 \pm 0.2 (3.5-4)	3.9 \pm 0.2 (3.6-4.3)	4.5 \pm 0.2 (37.1-48.3)	5.4 \pm 0.5 (4.6-6.4)	7.4 \pm 0.8 (6.1-9.1)	7.2 \pm 0.5 (6.5-8.1)
c	13.8 \pm 0.6 (12.9-14.8)	23.4 \pm 1.6 (20.4-26)	44.3 \pm 2.7 (39.8-48.8)	63.6 \pm 5.4 (52.0-71.9)	94.7 \pm 8.3 (79.9-113.5)	76.9 \pm 8.4 (64.7-92.9)
c'	4.18 \pm 0.20 (3.85-4.52)	2.28 \pm 0.20 (1.93-2.54)	1.20 \pm 0.06 (1.10-1.32)	0.93 \pm 0.07 (0.81-1.07)	0.71 \pm 0.04 (0.62-0.79)	0.84 \pm 0.06 (0.76-0.93)
d	5.9 \pm 0.32 (5.3-6.3)	6.7 \pm 0.55 (6.2-7.9)	7.6 \pm 0.47 (6.9-8.4)	8.0 \pm 0.53 (7.1-8.8)	8.6 \pm 0.39 (7.9-9.3)	8.1 \pm 0.40 (7.3-8.7)
d'	2.3 \pm 0.08 (2.1-2.3)	2.5 \pm 0.12 (2.3-2.8)	2.9 \pm 0.14 (2.7-3.2)	3.0 \pm 0.11 (2.8-3.3)	3.3 \pm 0.16 (3.1-3.6)	3.2 \pm 0.15 (2.9-3.5)
J	4.0 \pm 0.33 (3.6-4.6)	1.8 \pm 0.19 (1.3-2)	0.4 \pm 0.04 (0.3-0.5)	0.3 \pm 0.02 (0.3-0.4)	0.3 \pm 0.03 (0.3-0.4)	0.3 \pm 0.03 (0.3-0.4)
V	-	-	-	-	39.0 \pm 2.2 (35.6-43.6)	-
Odontostyle	61 \pm 3.5 (53-65)	74 \pm 1.9 (72-77)	95 \pm 3.7 (88-100)	117 \pm 3.1 (111-122)	139 \pm 5.8 (127-147)	137 \pm 4.2 (130-147)
Odontophore	39 \pm 1.5 (37-42)	48 \pm 2.2 (43-52)	60 \pm 4.5 (50-65)	71 \pm 3.3 (65-78)	84 \pm 2.2 (80-88)	82 \pm 2.5 (78-87)
Total stylet	100 \pm 3.8 (93-107)	123 \pm 3.9 (115-128)	156 \pm 4.6 (150-163)	188 \pm 5.5 (176-200)	223 \pm 6.3 (208-232)	219 \pm 4.8 (208-228)
Replacement odontostyle	75 \pm 2.6 (72-78)	94 \pm 3.7 (85-99)	116 \pm 4.2 (108-122)	140 \pm 4.0 (133-151)	-	-
Oral aperture to guide ring	49 \pm 1.4 (48-53)	65 \pm 3.1 (60-71)	83 \pm 2.9 (79-91)	100 \pm 4.1 (93-106)	1230 \pm 6.5 (115-142)	123 \pm 4.4 (115-131)
Tail	68 \pm 2.9 (64-71)	50.8 \pm 3.3 (46-57)	37 \pm 3.0 (31-43)	35 \pm 2.1 (30-40)	35 \pm 2.9 (30-40)	41.6 \pm 3.2 (37-47)
J (hyaline portion of tail)	26.8 \pm 2.4 (23.3-30.6)	19.3 \pm 1.9 (16.7-22.5)	7.8 \pm 0.7 (6.7-9.2)	7.8 \pm 1.0 (5.0-9.6)	10.7 \pm 1.0 (8.3-11.7)	9.2 \pm 0.9 (7.5-10.8)
Body diam. at lip region	8.4 \pm 0.4 (7.9-9.2)	9.7 \pm 0.5 (8.3-10)	10.9 \pm 0.7 (9.6-11.7)	12.6 \pm 0.6 (11.7-13.6)	15.1 \pm 0.4 (14.6-15.8)	15.2 \pm 0.4 (14.2-16.2)
Body diam. at guide ring	18.9 \pm 0.4 (18.3-19.4)	24.6 \pm 1.4 (22.2-27.5)	31.6 \pm 1.4 (29.3-34.6)	38.0 \pm 2.2 (34.2-42.5)	50.3 \pm 2.9 (45-55)	48.7 \pm 1.9 (45.0-52.5)
Body diam. at base of pharynx	24.1 \pm 1.2 (23.3-26.7)	30.6 \pm 2.1 (28.3-35.8)	37.8 \pm 2.8 (34.2-42.9)	45.8 \pm 3.7 (38.3-53.3)	61.4 \pm 4.8 (53.3-70)	61.0 \pm 3.6 (54.2-68.3)
Body diam. at mid-body or vulva	24.6 \pm 1.1 (23.3-26.7)	30.6 \pm 2.1 (28.3-35.8)	38.7 \pm 3.0 (35.0-45.8)	47.1 \pm 4.4 (38.3-56.7)	68.3 \pm 6.0 (57.9-78.3)	66.5 \pm 4.7 (55.8-75)
Body diam. at anus	16.2 \pm 1.0 (15.0-18.3)	22.4 \pm 1.4 (20.0-25.8)	30.7 \pm 1.8 (28.3-34.6)	37.7 \pm 2.2 (33.3-41.7)	49.4 \pm 2.1 (46.7-53.3)	49.3 \pm 1.9 (45.0-53.3)
Body diam. at beginning of J	6.7 \pm 0.5 (5.8-7.5)	11.0 \pm 1.5 (8.3-12.5)	18.0 \pm 1.3 (15.0-20)	23.7 \pm 2.5 (16.7-26.7)	32.9 \pm 2.3 (30.0-38.3)	27.8 \pm 1.7 (23.3-30.8)
Spicules	-	-	-	-	-	82.5 \pm 2.8 (77-87)
Lateral guiding piece	-	-	-	-	-	15.8 \pm 1.3 (13.3-18.3)

d, anterior to guide-ring/body width at lip region (Brown et al. 1994). d', body width at guide ring/body width at lip region (Brown et al. 1994). J, length of the hyaline region of the tail/hyaline width (Lišková et al. 1997).

Table 2. Morphometrics of juvenile stages of the *Xiphinema globosum* populations from Germany, Slovenia, Spain and Croatia in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Odontostyle (μm)	Replacement odontostyle (μm)	Oral aperture to guide ring (μm)	Tail length (μm)
J1						
Germany ¹	11	0.90 (0.78-1.00)	59 (56-64)	72 (64-76)	48 (45-53)	77 (71-80)
Slovenia ²	3	0.91 (0.87-0.97)	62.4 (60.3-64.1)	73.1 (71-75.4)	54.8 (54-56.5)	67.5 (64.2-72.8)
Italy ³	–	–	–	–	–	–
Spain ⁴	4	0.851 (0.81-0.92)	50 (48-52)	66 (61-69)	45 (43-47)	65 (61-70)
Croatia ⁵	7	0.94 (0.90-0.98)	61 (53-65)	75 (72-78)	49 (48-53)	68 (64-71)
Sum	25					
J2						
Germany ¹	7	1.29 (1.17-1.41)	74 (72-76)	93 (88-96)	60 (55-65)	59 (57-61)
Slovenia ²	4	1.3 (1.2-1.42)	73.8 (71.6-77.9)	96.7 (95.5-98)	69.7 (67.9-72.9)	55.9 (52.8-59)
Italy ³	–	–	–	–	–	–
Spain ⁴	3	1.157 (1.045-1.29)	63 (62-64)	89 (88-92)	52 (49-56)	53 (47-58)
Croatia ⁵	11	1.18 (1.07-1.28)	74 (72-77)	94 (85-99)	65 (60-71)	51 (46-57)
Sum	25					
J3						
Germany ¹	6	1.82 (1.52-2.06)	92 (89-95)	115 (108-119)	81 (70-89)	38 (36-42)
Slovenia ²	6	1.77 (1.65-1.94)	96.7 (95.5-99.3)	121.2 (116.9-124.4)	89 (80.4-95.5)	38.5 (36.1-40.2)
Italy ³	–	–	–	–	–	–
Spain ⁴	4	1.596 (1.545-1.645)	95 (89-99)	115 (112-116)	78 (70-87)	37 (33-41)
Croatia ⁵	12	1.62 (1.48-1.72)	95 (88-100)	116 (108-122)	83 (79-91)	37 (31-43)
Sum	28					
J4						
Germany ¹	4	2.41 (2.14-2.83)	118 (115-120)	140 (139-141)	100 (88-111)	35 (31-39)
Slovenia ²	7	2.36 (2.05-2.57)	121.2 (119.4-124.4)	147.4 (140.8-157.1)	113.7 (108.1-120)	35.3 (32.7-37.7)
Italy ³	–	–	–	–	–	–
Spain ⁴	6	2.236 (2.01-2.43)	114 (111-121)	141 (135-146)	95 (80-104)	37 (33-39)
Croatia ⁵	17	2.21 (1.82-2.55)	117 (111-122)	140 (133-151)	100 (93-106)	35 (30-40)
Sum	34					

¹Sturhan 1978; ²Barsi 1992; ³Roca and Lamberti 1994; ⁴Cantalapiedra-Navarrete et al. 2011; ⁵Original.

Table 3. Morphometrics of females and males of the *Xiphinema globosum* populations from Germany, Slovenia, Italy, Spain and Croatia in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Odontostyle (μm)	Tail length (μm)
Females				
Germany ¹	22	3.46 (2.63-3.93)	144 (132-152)	31 (27-34)
Slovenia ²	16	3.15 (2.85-3.45)	146.3 (140.1-155.9)	34.8 (28.9-39)
Italy ³	12	3.1 (2.8-3.5)	145.5 (129-154)	30.5 (26.5-34.5)
	5	3.2 (2.8-3.5)	145.5 (142-150)	31.5 (29-33)
Spain ⁴	10	2.978 (2.71-3.21)	138 (128-143)	35.5 (29-42)
Croatia ⁵	18	3.31 (2.85-3.64)	139 (127-147)	35 (30-40)
	Sum	83		
Males				
Germany ¹	4	3.27 (3.03-3.54)	142 (138-145)	38 (36-40)
Slovenia ²	9	3.1 (2.61-3.4)	146.8 (138.2-159.6)	39.2 (37.1-41.5)
Italy ³	12	3.1 (2.7-3.4)	145.5 (129-154)	35.5 (31-41)
	3	3.1 (3-3.4)	148.0 (144.5-153)	35.5 (32.5-40)
Spain ⁴	6	2.876 (2.645-3.46)	140 (137-145)	33.3 (31-35)
Croatia ⁵	18	3.17 (2.73-3.51)	137 (130-147)	42 (37-47)
	Sum	52		

¹Sturhan 1978; ²Barsi 1992; ³Roca and Lamberti 1994; ⁴Cantalapiedra-Navarrete et al. 2011; ⁵Original.

Table 4. Morphometrics of females of *Xiphinema globosum* populations from Germany, Slovenia, Italy, Spain and Croatia in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Total stylet (μm)	Oral aperture to guide ring (μm)	V (%)
Females					
Germany ¹	22	3.46 (2.63-3.93)	231 (219-241)	121 (111-141)	40.1 (37.6-43.8)
Slovenia ²	16	3.15 (2.85-3.45)	231.6 (225-237.6)	138.7 (129.5-146.4)	41.8 (40.8-43.3)
Italy ³	12	3.1 (2.8-3.5)	220 ⁶ (-)	133.0 (128-139.5)	41.5 (39.5-44.5)
	5	3.2 (2.8-3.5)	222.5 ⁶ (-)	133.0 (130-136)	41.0 (39.5-43)
Spain ⁴	10	2.978 (2.71-3.21)	219 ⁶ (-)	122 (102-139)	43.4 (41-47)
Croatia ⁵	18	3.31 (2.85-3.64)	223 (208-232)	130 (115-142)	39.0 (35.6-43.6)
Sum		83			

¹Sturhan 1978; ²Barsi 1992; ³Roca and Lamberti 1994; ⁴Cantalapiedra-Navarrete et al. 2011; ⁵Original; ⁶Sum of average values of odontostyle and odontophore lengths.

Table 5. Morphometrics of males of *Xiphinema globosum* populations from Germany, Slovenia, Italy, Spain and Croatia in the form mean (range).

Developmental stages and populations	No. of specimens	Body length (mm)	Total stylet (μm)	Oral aperture to guide ring (μm)	Spicule (μm)	Lateral guiding piece (μm)
Males						
Germany ¹	4	3.27 (3.03-3.54)	228 (225-232)	118 (111-128)	74.5 (72-77)	18 (17-19)
Slovenia ²	9	3.1 (2.61-3.4)	230.8 (223.7-241.3)	134.7 (127-143.9)	84.5 (80-85.5)	18.5 (16.5-20)
Italy ³	12	3.1 (2.7-3.4)	219 ⁶ (-)	129.0 (116.5-139)	83.5 (76.9-91.2)	17 (14.2-20)
	3	3.1 (3-3.4)	219.5 ⁶ (-)	130.0 (126.5-132)	88.0 (80-90)	15.5 (13.5-17)
Spain ⁴	6	2.876 (2.645-3.46)	219 ⁶ (-)	127 (117-136)	75 (70-80)	20.2 (18-21)
Croatia ⁵	18	3.17 (2.73-3.51)	219 (208-228)	123 (115-131)	82.5 (77-87)	15.8 (13.3-18.3)
Sum		52				

¹Sturhan 1978; ²Barsi 1992; ³Roca and Lamberti 1994; ⁴Cantalapiedra-Navarrete et al. 2011; ⁵Original; ⁶Sum of average values of odontostyle and odontophore lengths.